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Sanitation of Styroblocks to Control Algae and Seedling Root Rot Fungi

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Sanitation of Styroblocks to Control Algae and Seedling Root Rot Fungi

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ABSTRACT

The purpose of this study was to determine ways of cleaning used styroblocks, the styrofoam containers in which forest seedlings are produced. Experiments were conducted in three trials where compounds or methods were examined for their sanitizing efficacy.

The most effective methods for reducing fungal and algal propagules on used containers were: steam (95°C for 1 minute); heated soaps (Safer's *De-moss* and *Ivory* soap, 10-second dip in 5% solution at 80°C); bleach (10-second dip in a 0.5% solution buffered to pH 7.0); hydrogen peroxide (10-second dip in 10% solution); sodium metabisulphite (10-second dip in 5% solution); and sulphur dioxide fumigation.

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INTRODUCTION

Most forest nursery seedlings in British Columbia are grown in containers molded from expanded polystyrene beads (styroblocks). With repeated use these styroblocks deteriorate. Cracks and holes develop and the container surfaces accumulate root pieces and other organic material. Pathogenic fungi such as *Fusarium*, *Phoma*, *Cylindrocarpon*, and *Pythium* spp. colonize the used containers and cause seedling root rots (James *et al.* 1988; Sturrock and Dennis 1988; James 1989). Algae also occur on container surfaces and impede seedling production by preventing penetration of water and nutrients into the growing medium.

Increased container seedling production overall, particularly of 2+0 seedlings, is resulting in seedling roots undergoing longer periods of exposure to styroblock-borne pathogens. This, in addition to the expense of new containers, is the reason effective methods are needed for sanitizing containers between crops.

Styroblocks have been conventionally cleaned with a high pressure water wash to remove surface adherents. One negative effect of this, however, is the erosion of the container surface, contributing to its ability to accumulate fungus propagules. After washing, the containers are routinely rinsed in a sterilizing solution, such as sodium hypochlorite (household bleach), sodium metabisulphite, soaps, and algicides. Continued losses of seedlings to root rots, as well as the increase in root rot incidence with styroblock age, indicate that these cleaning procedures are only partially effective.

Treatment of seedling containers to free them of pathogenic organisms nas long been an accepted procedure (Baker *et al.* 1957). Both heat and chemical treatments are used. Heat can be applied as steam, hot water baths, or microwaves (Moore *et al.* 1983). James and Woollen (1989) significantly reduced the occurrence of *Fusarium* and *Cylindrocarpon* spp. on styroblocks by soaking them in hot water (68°C for 10 minutes). This supports the findings of Sturrock and Dennis (1988) who eliminated *Fusarium*, *Cylindrocarpon*, and *Pythium* on styroblocks by immersing them in 80°C water for 3 minutes.

Chemical treatments of containers are varied and include fumigation and rinsing in chemical solutions. Methyl bromide has been used to sanitize containers at the same dosages (1.8 kg/2.8 m^3) used to fumigate soil (Baker *et al.* 1957). However, Ebben *et al.* (1983) found that some concentrations of methyl bromide, normally toxic for soil fumigation, did not kill several important soil-borne pathogens, including *Fusarium oxysporum* and *F. culmorum*. Formaldehyde is useful as a rapid disinfectant for tools and may be of benefit as a container sterilant (Baker *et al.* 1957). Copper compounds (e.g. copper naphthenate) are excellent materials for treating wooden containers (Baker *et al.* 1957). These compounds often persist for up to a year, killing pathogens near the container surface by slowly leaching into the soil for a distance of 5–10 cm. Injury to roots in this zone is often seen as darkening or killing of the tips.

Algicides containing bromine compounds are effective for sanitizing floriculture equipment, killing a variety of algae, fungi, and bacteria (Tayama *et al.* 1986). In a 1986 production information bulletin, the Great Lakes Chemical Corporation (West Lafayette, Ind.) claimed that many of these compounds display little toxicity to flowers or foliage.

Two compounds used by the food industry as sterilants are iodine and sodium metabisulphite. Plastic containers used for commercial lettuce production in Britain are effectively sanitized in an iodine rinse following a high pressure hot water wash (Lawson 1989). Acceptable container sanitation is achieved in Britain in 0.3% iodine solutions (H. Meijer, pers. comm., Mafo Howden, Oldenzaal, Netherlands, 1989). Anhydrous sodium metabisulphite (ABS) is used in the brewing process as a sterilant and a chemical to kill naturally occurring yeasts. It has also been effective for reducing *Fusarium* spp. on styroblock containers (Sturrock and Dennis 1988). When mixed with water, ABS releases sulphur dioxide (SO_2) at a rate that increases with temperature.

Chlorine compounds such as sodium hypochlorite or solid calcium hypochlorite, used to sterilize water, are also used in solution to clean equipment. To be most effective, the Florida Co-operative

Extension Service (Hicks and Segall 1981) recommends buffering these solutions to a pH of between 6.0 and 7.5. When household bleach is dissolved in water it forms hypochlorous acid (HOCl). The sanitizing action occurs as oxygen is released in the breakdown of HOCl into HCl and O_2. This occurs best at a pH of 6.0–7.5. Above a pH of 7.7, hypochlorite ions (OCl^-) become dominant and have much less killing power than hypochlorous acid. Ozone (O_3) used to treat domestic water supplies, is an effective sterilant against both fungi and bacteria (Evans 1972).

Wetting agents improve the solution penetration of pathogen lesions. Surfactants, large molecule, sticking, or spreading agents lower surface tension of spray liquids. Wetting agents, and surfactants are both used to enhance the activity of sterilants. Surfactants, for example, increased the effectiveness of disinfectants used to control decay fungi on pears (Spotts and Cervantes 1989). This enhanced activity may also be evident with soaps, including quaternary ammonium compounds. Sturrock and Dennis (1988) found a heated soap solution to be effective at controlling fungi and algae on styroblocks.

Electromagnetic and electron beam radiation are other treatments that kill fungal spores. Short wavelength (254 nm) ultraviolet (UV) radiation is a well-known fungicidal agent (Rotem *et al.* 1985). Electron beam radiation produced by high-energy electron, linear accelerators reduced wood chip decay from 2.4 to 0.4% by altering the temperature profile within the pile, thus preventing inoculation from airborne *Poria monticola* spores (S. Hyndman, pers. comm., Hyndman International Ltd, Vancouver, 1989).

This study addresses the problem of root rot fungi and algae build-up from season to season. The experiments used were designed to assess the effectiveness of the above materials and methods for sanitizing container-nursery styroblocks. The work expands on that done in the styroblock sanitation trials conducted by the B. C. Ministry of Forests, Forestry Canada, and private nurseries in British Columbia (Sturrock and Dennis 1988). The original trials were intended to address concerns over moss and algae build-up on used styroblocks, but laboratory assays by Sturrock and Dennis (1988) showed that inoculum of root rot fungi such as *Fusarium* spp. survived even rigorous container washing. In this study, three trials were carried out. The first two were used to identify the most effective styroblock sanitizing methods. The best of these were examined in a third trial, and the phytotoxicity of the treatments was assessed.

METHODS

Used styroblocks from several nurseries on Vancouver Island were assayed to confirm the presence of pathogenic fungi and algae on them. *Fusarium*, *Phoma*, and *Cylindrocarpon* fungal spp., as well as algae, were found on one source of containers. Styroblocks from this source were then used in three trials to assess container sanitization. In the first two trials, 10 styroblocks were randomly assigned to each treatment, with one container constituting a replicate. Before being treated, each styroblock received a high pressure water wash (40°C at 7 kg/cm^2). Chemical rinse treatments were conducted in an aluminium tank accommodating one styroblock. Rinse solutions were heated with a propane torch applied to a series of copper coils in the tank bottom. Unless noted, solution temperatures were maintained at 15°C with an exposure time of 10 seconds. Treatments other than rinsing are noted as they occurred. Immediately following each treatment, an electric jigsaw was used to cut twenty 60 X 10 mm pieces of styroblock from the upper surface of 20 randomly selected cavities per styroblock, for a total of 200 samples per treatment. The saw blade was cleaned with 100% ethyl alcohol after each sample was cut.

The interior cavity surface of each styrofoam piece was pressed onto soil algae agar (SAA) (Poindexter 1971). These were incubated for 30 days at 22°C under prevailing daylight. Algae presence was then determined, with each styroblock piece supporting algal growth being counted as one. Twenty pieces of styroblock were cut from the lower surface of 20 randomly selected cavities per styroblock, for a total of 200 samples per treatment. These pieces were plated on a *Fusarium* selective medium (KM) (Komada 1975), in the same manner as those sampled from the cavity's upper surfaces. These samples

were incubated for 7–14 days at 22°C under prevailing daylight. Fungi growing from the styroblock pieces were identified by genus, with each styroblock piece supporting a particular fungal growth being counted as one colony for that genus.

Trial 1

Trial 1 tested 17 compounds and sanitizing methods. Treatments varied by concentration, temperature, and time of exposure, for a total of 34 treatments (Table 1).

TABLE 1. Trial 1 treatments. Unless otherwise noted, solution temperatures were maintained at 15°C with an exposure time of 10 seconds. Treatments other than rinsing are noted.

Treatment	Compound or sanitizing method	Concentration, temperature, and time of exposure[a]
1.1a	Control	High pressure wash before H_2O dip
1.2a	Control	No high pressure wash before H_2O dip
2.1a	RX44(quaternary ammonium)	1.5% solution
3.1a	*Bactrex*	9.0% solution
4.1a	*Roccal*	0.8% solution
5.1a	*Lysoquat*	0.4% solution
6.1a	*Lysofume* (formaldehyde)	0.8% solution
7.1a	*Lysoquat* + *Lysofume*	0.4% + 0.8% solution
8.1a	Borax	4.0% solution
9.1a	Bleach	0.5% solution
9.2a	Bleach	0.5% solution buffered to pH 7.0
9.3a	Bleach	Dip in 5% wetting agent (Tween 20) before dip in 0.5% bleach
9.4a	Bleach	Dip in 5% glucose solution followed by 4-day incubation at 20°C before dip in 0.5% bleach
10.1a	Sodium metabisulphite	2.0% solution
10.2a	Sodium metabisulphite	5.0% solution
11.1a	*De-Moss* (Safer's soap)	5.0% solution at 15°C
11.2a	*De-Moss*	5.0% solution at 30°C
11.3a	*De-Moss*	5.0% solution at 50°C
11.4a	*De-Moss*	5.0% solution at 80°C
12.1a	*Ivory* soap	5.0% solution at 15°C
12.2a	*Ivory* soap	5.0% solution at 30°C
12.3a	*Ivory* soap	5.0% solution at 50°C
12.4a	*Ivory* soap	5.0% solution at 80°C
13.1a	Hydrogen peroxide	30% solution
14.1a	Ultraviolet radiation	2.73 W/m^2 at 348 nm for 1 minute
14.2a	Ultraviolet radiation	2.73 W/m^2 at 348 nm for 2 minutes
14.3a	Ultraviolet radiation	2.73 W/m^2 at 348 nm for 3 minutes
14.4a	Ultraviolet radiation	2.73 W/m^2 at 348 nm for 4 minutes
15.1a	Microwave radiation	700 W for 2 minutes
16.1a	Ozone	Saturated H_2O solution 1 minute
16.2a	Ozone	Saturated H_2O solution 2 minutes
16.3a	Ozone	Saturated H_2O solution 3 minutes
16.4a	Ozone	100% O_3 gas at 30°C 3 minutes
17.1a	*Bro-mo-gas*	70 cc methyl bromide/2.83 m^3 for 24 h

[a] Unless otherwise noted, each treatment received an initial high pressure water wash.

Trial 2

A second trial tested 14 compounds and sanitizing methods. Several methods found effective in trial 1 were examined in greater detail. Treatments again varied by concentration, temperature, and time of exposure, for a total of 60 treatments (Table 2).

TABLE 2. Trial 2 treatments. Unless otherwise noted, solution temperatures were maintained at 15°C with an exposure time of 10 seconds. Treatments other than rinsing are noted.

Treatment	Compound or sanitizing method	Concentration, temperature, and time of exposure[a]
1.1b	Control	High pressure water wash before H_2O dip at 15°C
1.2b	Control	30°C
1.3b	Control	50°C
1.4b	Control	80°C
2.1b	Control	No high pressure water wash before H_2O dip at 15°C
2.2b	Control	30°C
2.3b	Control	50°C
2.4b	Control	80°C
3.1b	*Lysoquat*	0.4% solution
4.1b	Bleach	5.0% solution buffered to pH 7.0
4.2b	Bleach	0.5% solution at 30°C
4.3b	Bleach	50°C
4.4b	Bleach	80°C
4.5b	Bleach	High pressure water wash before dip in 5.0% *Ivory* soap followed by dip in 0.5% bleach
4.6b	Bleach	No high pressure water wash before dip in 5.0% *Ivory* soap followed by dip in 0.5% bleach
5.1b	Sodium metabisulphite	0.5% solution
5.2b	Sodium metabisulphite	1.0% solution
5.3b	Sodium metabisulphite	Dip in 5.0% *Ivory* soap followed by dip in 0.5% sodium metabisulphite
5.4b	Sodium metabisulphite	Dip in 5.0% *Ivory* soap followed by dip in 1.0% sodium metabisulphite
5.5b	Sodium metabisulphite	Dip in 5.0% *Ivory* soap followed by dip in 2.0% sodium metabisulphite
6.1b	*Enhance* (sodium bromide)	Bromine solution levels maintained at 5 ppm
6.2b	*Enhance* (algicide)	7 ppm
6.3b	*Enhance*	10 ppm
6.4b	*Enhance*	12 ppm
6.5b	*Enhance*	15 ppm
7.1b	*Algymycin*	30 ppm; 0.89 ppm Cu
7.2b	*Algymycin*	150 ppm; 0.95 ppm Cu
7.3b	*Algymycin*	210 ppm; 1.3 ppm Cu
7.4b	*Algymycin*	300 ppm; 1.9 ppm Cu
8.1b	*De-Moss* (Safer's soap)	5.0% solution at 15°C
8.2b	*De-Moss*	30°C
8.3b	*De-Moss*	50°C
8.4b	*De-Moss*	80°C
9.1b	Hydrogen peroxide	3.0% solution
9.2b	Hydrogen peroxide	9.0% solution
9.3b	Hydrogen peroxide	12.0% solution
9.4b	Hydrogen peroxide	15.0% solution
9.5b	Hydrogen peroxide	20.0% solution
9.6b	Hydrogen peroxide	30.0% solution
10.1b	Iodine	0.3% solution
10.2b	Iodine	1.5% solution
10.3b	Iodine	3.0% solution

TABLE 2. (Continued)

Treatment	Compound or sanitizing method	Concentration, temperature, and time of exposure[a]
11.1b	Steam	80°C for 3 minutes
11.2	Steam	90°C for 1 minute
11.3b	Steam	90°C for 3 minutes
11.4b	Steam	100°C for 1 minute
12.1b	Electron beam radiation	0.15 Megarad
12.2b	Electron beam radiation	0.25 MRad
12.3b	Electron beam radiation	0.6 MRad
12.4b	Electron beam radiation	1.1 MRad
12.5b	Electron beam radiation	3.2 MRad
12.6b	Electron beam radiation	5.0 MRad
13.1b	Ultraviolet radiation	2.73 W/m^2 348 nm-1 minute
13.2b	Ultraviolet radiation	2.73 W/m^2 348 nm-2 minutes
13.3b	Ultraviolet radiation	2.73 W/m^2 348 nm-4 minutes
14.1b	Microwave radiation	700 W for 2 minutes
14.2b	Microwave radiation	700 W for 3 minutes
14.3b	Microwave radiation	700 W for 4 minutes
14.4b	Microwave radiation	700 W for 5 minutes

[a] Unless otherwise noted, each treatment received an initial high pressure water wash.

Trial 3

Trial 2 treatments effective at controlling fungi and algae, and being practical to implement operationally, were studied in a third trial made up of 10 treatments (Table 3). A water control heated to 80°C was added to trial 3 for comparison to both heated soap treatments. Sulphur dioxide gas was included in trial 3 as a fumigant. Treatment phytotoxicity was also assessed. Following treatment, each styroblock was cut in half. One half of each was used to assess treatment efficacy for controlling fungi and algae as outlined for trials 1 and 2; the other half was filled with peat and 28 cavities were sown with lettuce at a rate of one seed per cavity. Sturrock and Dennis (1988) used lettuce to study the phytotoxic effects of ABS, and it was chosen for this study because of its rapid germination and growth rate. The lettuce was watered daily and incubated at 25°C for 5 weeks under prevailing daylight. Numbers of surviving plants were counted each week and, at the end of the observation period, plant fresh weights and oven-dried (60°C for 24 hours) weights were recorded.

TABLE 3. Trial 3 treatments. Unless otherwise noted, solution temperatures were maintained at 15°C with an exposure time of 10 seconds. Treatments other than rinsing are noted.

Treatment	Compound or sanitizing method	Concentration, temperature, and time of exposure[a]
1.1c	Control	H_2O dip at 15°C
1.2c	Control	H_2O dip at 80°C
2.1c	*De-Moss*	5.0% solution at 80°C
3.1c	*Ivory* soap	5.0% solution at 80°C
4.1c	Bleach	0.5% solution buffered to pH 7.0
5.1c	Sodium metabisulphite	5.0 % solution
6.1c	Hydrogen peroxide	10.0% solution
7.1c	Steam	95°C for 1 minute
8.1c	Iodine	3.0% solution
9.1c	Sulphur dioxide	100% SO_2 gas at 15°C for 24 hours

[a] Each treatment received an initial high pressure water wash.

Data Analysis

Treatment efficacy

Pieces of styroblock supporting fungal or algal growth in culture were counted for each treatment and grouped to include all species of fungi and algae combined, all species of fungi combined, individual fungal species (i.e., *Fusarium*, *Phoma*, and *Cylindrocarpon*), and algae. Numbers of colonized styroblock pieces were then used to sort treatments from each trial in order of increasing effectiveness. These were expressed as a percent of the total number of colonies possible (i.e., 200 samples/treatment). Further analysis was limited to 10 treatments from trials 1 and 2 and all treatments from trial 3.

Treatments were selected on the basis of their ranked effectiveness and ease of operational application. These data were then subjected to an arcsin transformation, after which chi-square analysis showed them to satisfy the hypothesis of homogeneity. Duncan's multiple range analysis (MRA) was performed on the transformed data for all species of fungi and algae combined, all species of fungi combined, individual fungal species, and all algae.

Treatment phytotoxicity

To assess treatment phytotoxicity, Duncan's MRA was performed on the arcsin-transformed number of lettuce survivors per treatment, plant fresh weights per treatment, and oven-dried weights per treatment.

RESULTS

Treatments from trials 1, 2, and 3 are listed in Appendix 1 in order of increasing effectiveness for all species of fungi and algae combined. The 10 treatments selected from trials 1 and 2 for inclusion in the MRA are compared in Appendix 2. Duncan's MRA for all treatments in trial 3 is shown in Appendix 3.

Of the 10 treatments from trials 1 and 2 (Figures 1 and 2), 30% hydrogen peroxide and heat applied in the form of steam were the most effective at controlling fungi and algae in general. Hydrogen peroxide and steam were also the only treatments that proved to be effective in trials 1 and 2 when algae alone were considered (Figures 3 and 4). The species of fungi most tolerant of any of the treatments in trials 1 and 2 was *Phoma*, but the algae were more resistant to treatment than any of the fungi (Figures 3 and 4).

Figure 5 shows the mean percentage of trial 3 styroblock pieces supporting growth of any fungal or algal species combined and growth of any fungal species alone. Figure 6 shows trial 3 treatment efficacy for individual fungal species and algae. The treatment showing the greatest phytotoxic effect was iodine, as indicated by the mean number of surviving plants per styroblock (Appendix 4) and the comparison of mean fresh and oven-dried weights (Figure 7).

DISCUSSION

The results of this study indicate that several methods are effective for reducing populations of common root rot pathogens and algae (Appendices 1-3). However, although over 100 styroblock sanitizing treatments were examined in the 3 trials, no single compound could be identified as being effective for all species of fungi and algae. This finding underscores the diligence nursery growers must apply to container sanitation if they want to minimize seedling losses to root rots. Growers must consider the history of disease and algae problems at their nursery; the costs of using a new system or modifying the existing one; and the environmental and human pros and cons that each treatment might carry. Growers can then compare the effectiveness of the treatments examined in trial 3 and select a sanitizing method best suited to their needs.

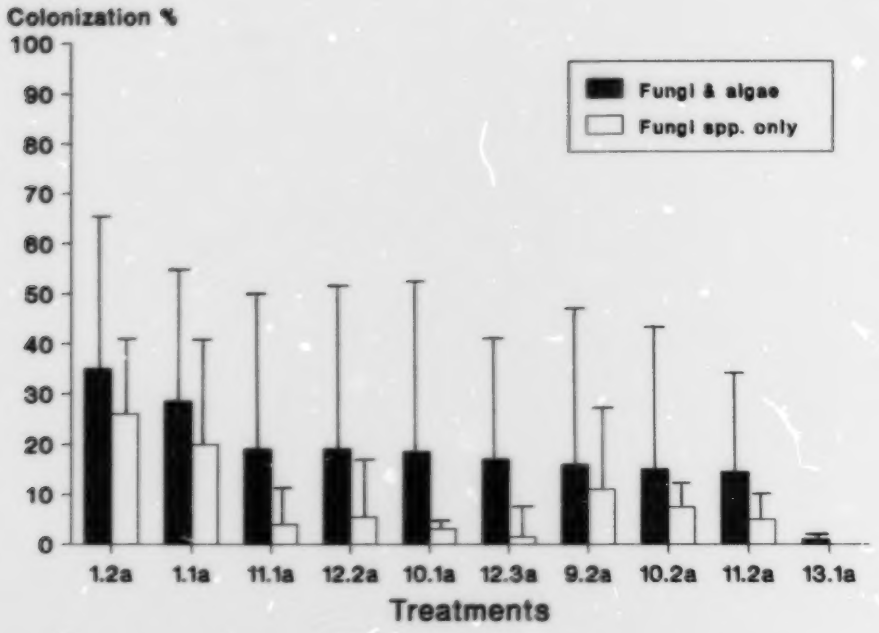

FIGURE 1. The mean percentage of styroblock pieces supporting fungal and algal growth and growth of fungal species only from trial 1. Vertical lines represent 1 standard deviation above the mean.

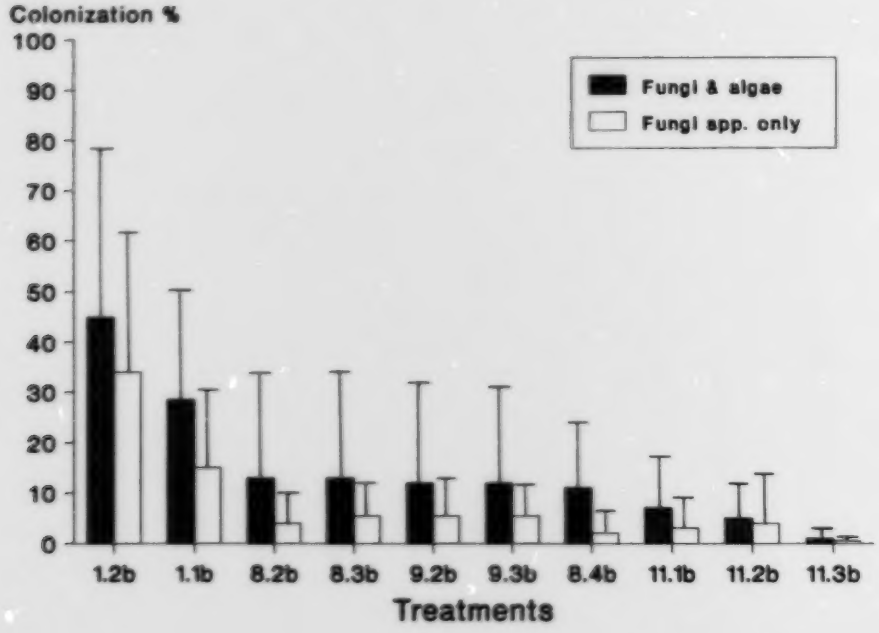

FIGURE 2. The mean percentage of styroblock pieces supporting fungal and algal growth and growth of fungal species only from trial 2. Vertical lines represent 1 standard deviation above the mean.

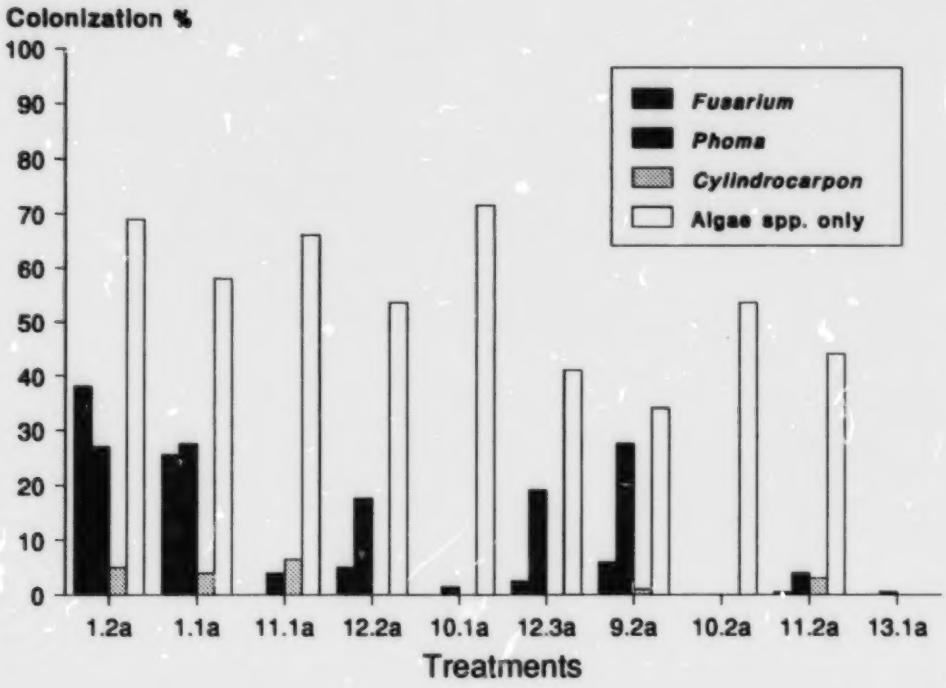

FIGURE 3. The mean percentage of styroblock pieces supporting individual fungal species and algal growth from trial 1.

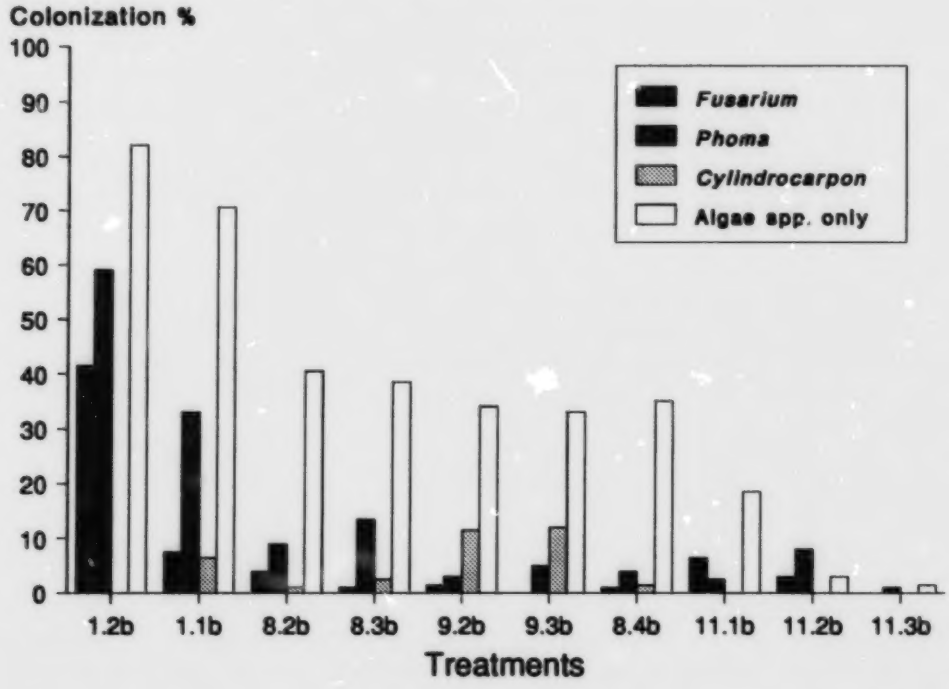

FIGURE 4. The mean percentage of styroblock pieces supporting individual fungal species and algal growth from trial 2.

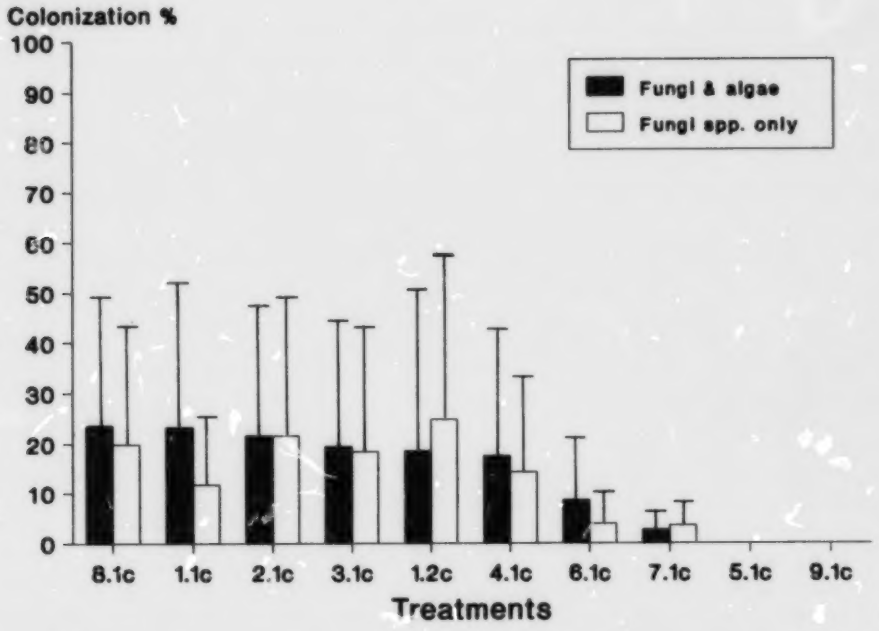

FIGURE 5. The mean percentage of styroblock pieces supporting fungal and algal growth and growth of fungal species only from trial 3. Vertical lines represent 1 standard deviation above the mean.

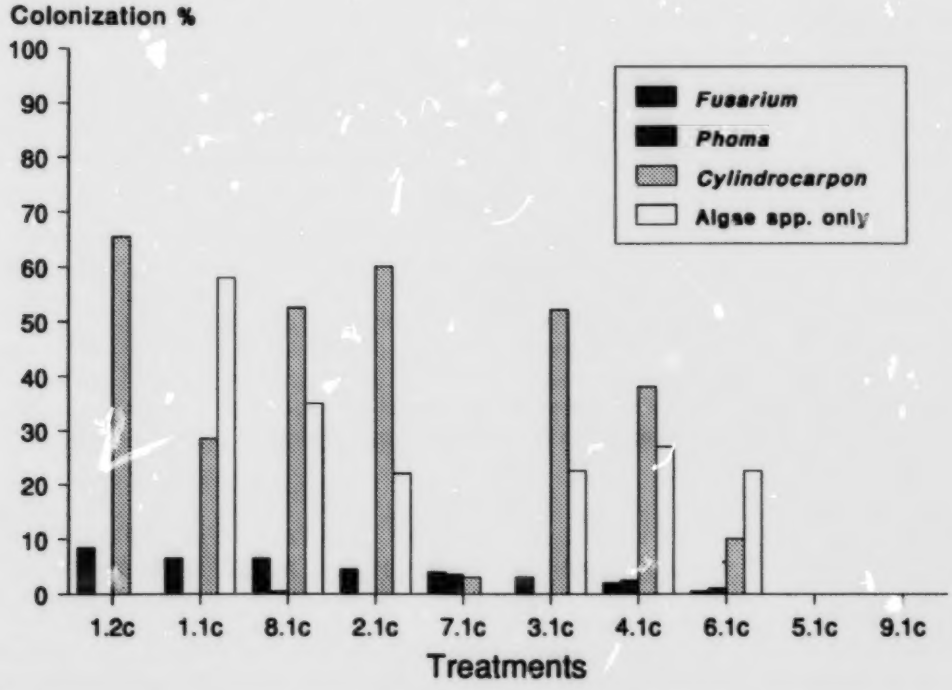

FIGURE 6. The mean percentage of styroblock pieces supporting individual fungal species and algal growth from trial 3.

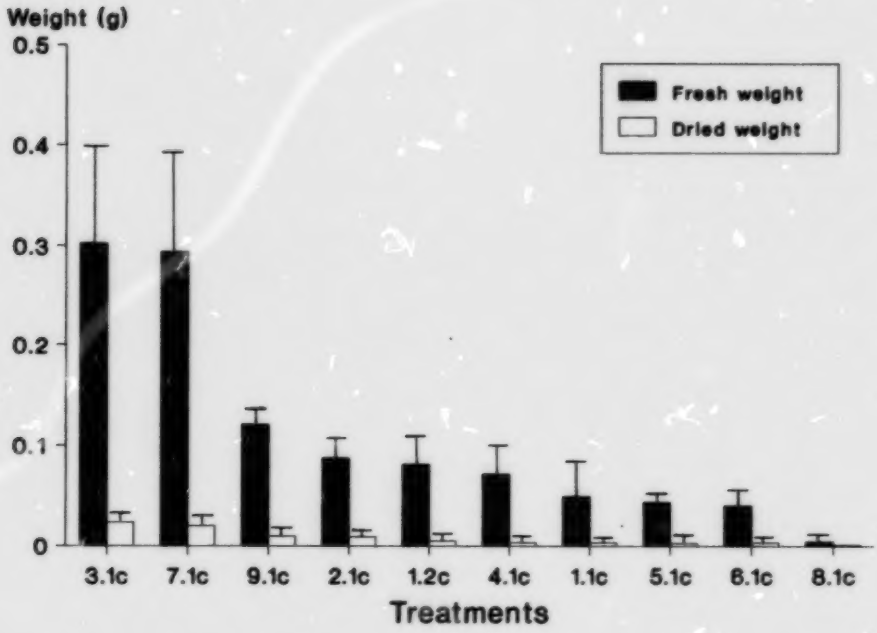

FIGURE 7. Fresh and oven-dried weights of lettuce plants grown in cavities of styroblocks treated in trial 3. Vertical lines represent 1 standard deviation above the mean.

On average, the three species of fungi examined appeared to be more susceptible to the treatments than were algae. This was even the case with the algicides examined, including Safer's *De-Moss*, a product formulated to control mosses and algae. If controlling algae is of more concern to a grower than controlling fungal populations, we recommend that steam sterilization be used. This treatment has several drawbacks, however. One is that a facility to steam clean styroblocks in large quantities is expensive to construct. Another is that steam causes distortion to styroblocks if it is injected rapidly and directly onto the containers, and if the containers are left in high heat (> 95°C) for a long period of time.

In addition to steam sterilization, several of the hot water treatments proved effective for reducing fungal and algal populations. This was especially so for the hot solutions that were soap-based. In trial 1, 15°C and 30°C *Ivory* soap solutions were as effective at controlling fungi and algae as were solutions of 15°C and 30°C Safer's *De-Moss* (Figure 1). However, *Ivory* soap was not that effective for *Phoma* spp. (Figure 3). In trial 2, *De-Moss* at 30°C and 50°C did not effectively control *Phoma* spp., but at 80°C it reduced all fungal populations (Figures 2 and 4). In trial 3, both *Ivory* soap and Safer's *De-Moss* at 80°C were effective at controlling fungi, but less so for *Cylindrocarpon* spp. Mean numbers of surviving lettuce plants in the trial 3 phytotoxicity tests indicate that both soap products show little phytotoxicity toward lettuce (Appendix 4).

If heated water, soap, or steam are too difficult for growers to use, chemical treatment might be considered. For example, a 0.5% (5000 ppm) sodium hypochlorite (household bleach) solution was effective in all three trials, but only when buffered with phosphoric acid and sodium hydroxide to a pH of 7.0. This supports the results of Hicks and Segall (1981) who recommend buffering bleach to facilitate the formation of hypochlorous acid. It would not be difficult for nursery personnel to monitor the pH of their bleach rinse solution and adjust it as necessary. The results of trials 1 and 2 indicate that failure to buffer bleach to a neutral pH makes it almost useless as a styroblock sterilant. Buffered bleach was not apparently phytotoxic to lettuce. Bleach solutions were heated in trial 2, but tests with a multi-gas detector (Drager, Lubeck, Federal Republic of Germany) showed that the chlorine gas content 1 m above the rinse

tank exceeded the permissible concentrations of the Worker's Compensation Board of British Columbia, for 8-hour exposures (Worker's Compensation Board of British Columbia 1980).

One effective treatment for sanitizing styroblocks is a 10% hydrogen peroxide solution (Figures 5 and 6). It effectively reduced fungal and algal populations, did not result in phytotoxicity to lettuce, and broke down to harmless H_2O. Nevertheless, its use has some drawbacks: it is expensive, difficult to handle, and extremely corrosive to all proteins (even 10% solutions must be handled with extreme caution by workers).

The two compounds most effective at controlling fungal and algal growth on styroblocks were a 5% sodium metabisulphite solution and sulphur dioxide fumigation (Figures 5 and 6). However, if sulphur dioxide is too expensive or logistically difficult for nursery growers to use, then we recommend using a 5% sodium metabisulphite rinse solution, followed by prompt containment of the styroblocks in plastic wrap or under a tarpaulin. At solution concentrations of 5%, sodium metabisulphite is safe to handle and appears to be non-phytotoxic to lettuce. Sturrock and Dennis (1988) recognized sodium metabisulphite to be effective for cleaning containers and it is commonly used safely in the brewing industry.

Another compound reported to be effective for cleaning lettuce containers is iodine (Lawson 1989). In this study, a 3% iodine solution was effective at reducing *Fusarium* and *Phoma* populations, but was also phytotoxic to lettuce. Not only were the number of surviving lettuce plants grown in the iodine-treated containers notably reduced compared to those plants grown in other containers, the plants were severely stunted, had very deformed leaves, and were covered with dark brown lesions. Their reduction in size is shown by their fresh and oven-dried weights (Figure 7). Iodine has been effectively used to sanitize hard plastic containers, but not styrofoam. Styroblocks may retain iodine and release it over time, thus resulting in a phytotoxic reaction.

CONCLUSIONS

The results of this study support the use of several compounds or methods for sanitizing styroblocks. Although no method is going to eradicate all algae or fungal propagules, growers can reduce the chances of encountering severe root rot problems by choosing from the following methods, the one best suited to their operation:

- Heat treatments
  - Steam 95°C for 1 minute
  - Heated soaps

Safer's *De-Moss*	5% solution at 80°C
Ivory soap	5% solution at 80°C

- Chemical treatments
  - Bleach 0.5% solution buffered to pH 7.0
  - Hydrogen peroxide 10% solution
  - Sodium metabisulphite 5% solution
  - Sulphur dioxide fumigation

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APPENDIX 1. Treatments listed in order of increasing effectiveness[a]

Trial 1

Treatment	Compound or sanitizing method	Mean percentage of styroblock pieces supporting fungal and algal growth
2.1a	RX44	46.5
9.1a	Bleach	40.6
16.2a	Ozone	39.9
8.1a	Borax	39.3
16.4a	Ozone	36.4
3.1a	*Bactrex*	34.5
1.2a	Control	34.5
9.3a	Bleach	34.4
17.1a	*Bro-mo-gas*	32.8
16.3a	Ozone	32.5
12.4a	*Ivory* soap	30.9
16.1a	Ozone	30.0
1.1a	Control	28.6
4.1a	*Roccal*	27.3
15.1a	Microwave radiation	25.0
12.1a	*Ivory* soap	24.3
6.1a	*Lysofume*	24.0
11.3a	Safer's *De-Moss*	23.6
11.4a	Safer's *De-Moss*	23.6
7.1a	*Lysoquat* + *Lysofume*	23.2
14.2a	Ultraviolet radiation	21.9
5.1a	*Lysoquat*	20.9
14.3a	Ultraviolet radiation	20.6
11.1a	Safer's *De-Moss*	19.1
12.2a	*Ivory* soap	19.0
10.1a	Sodium metabisulphite	18.3
12.3a	*Ivory* soap	15.6
9.2a	Bleach	15.0
10.2a	Sodium metabisulphite	13.4
11.2a	Safer's *De-Moss*	12.9
14.4a	Ultraviolet radiation	9.4
14.1a	Ultraviolet radiation	8.8
9.4a	Bleach	8.2
13.1a	Hydrogen peroxide	0.1

[a] Increasing treatment effectiveness for all species of fungi and algae combined.

Trial 2

Treatment	Compound or sanitizing method	Mean percentage of styroblock pieces supporting fungal and algal growth
14.3b	Microwave radiation	49.5
2.1b	Control	45.5
12.1b	Electron beam radiation	45.0
14.2b	Microwave radiation	44.0
4.2b	Bleach	44.0
13.1b	Ultraviolet radiation	43.5
13.2b	Ultraviolet radiation	43.5
4.6b	Bleach	43.5
4.3b	Bleach	42.0
14.1b	Microwave radiation	42.0
5.4b	Sodium metabisulphite	41.5
2.2b	Control	41.5
13.3b	Ultraviolet radiation	41.0
5.3b	Sodium metabisulphite	39.5
5.1b	Sodium metabisulphite	37.0
14.4b	Microwave radiation	36.0
12.2b	Electron beam radiation	35.0
10.2b	Iodine	33.5
4.4b	Bleach	32.5
5.2b	Sodium metabisulphite	32.0
2.3b	Control	31.5
4.5b	Bleach	31.0
1.3b	Control	30.0
1.1b	Control	29.5
7.2b	*Algymycin*	29.0
7.1b	*Algymycin*	28.5
6.5b	*Enhance*	28.5
10.1b	Iodine	28.0
7.4b	*Algymycin*	27.0
1.2b	Control	27.0
10.3b	Iodine	26.5
7.3b	*Algymycin*	25.0
6.1b	*Enhance*	24.0
6.3b	*Enhance*	24.0
3.1b	*Lysoquat*	22.5
6.4b	*Enhance*	21.5
8.1b	*De-Moss*	20.0
1.4b	Control	19.0
4.1b	Bleach	18.5
5.5b	Sodium metabisulphite	18.0
12.3b	Electron beam radiation	16.0
6.2b	*Enhance*	15.5
9.1b	Hydrogen peroxide	15.5
2.4b	Control	15.0
8.2b	*De-Moss*	14.0
8.3b	*De-Moss*	13.5
9.4b	Hydrogen peroxide	13.5
9.2b	Hydrogen peroxide	12.5

Trial 2 (Continued)

Treatment	Compound or sanitizing method	Mean percentage of styroblock pieces supporting fungal and algal growth
9.3b	Hydrogen peroxide	12.5
8.4b	*De-Moss*	10.5
11.1b	Steam	6.0
9.5b	Hydrogen peroxide	6.0
12.4b	Electron beam radiation	5.0
12.5b	Electron beam radiation	4.0
12.6b	Electronbeam radiation	4.0
11.2b	Steam	3.5
11.4b	Steam	2.5
9.6b	Hydrogen peroxide	0.5
11.3b	Steam	0.5

[a] Increasing treatment effectiveness for all species of fungi and algae combined.

Trial 3

Treatment	Compound or sanitizing method	Mean percentage of styroblock pieces supporting fungal and algal growth
8.1c	Iodine	23.6
1.1c	Control	23.3
2.1c	*De-Moss*	21.6
3.1c	*Ivory* soap	19.4
1.2c	Control	18.5
4.1c	Bleach	17.4
6.1c	Hydrogen peroxide	8.5
7.1c	Steam	2.6
5.1c	Sodium metabisulphite	0.0
9.1c	Sulphur dioxide	0.0

[a] Increasing treatment effectiveness for all species of fungi and algae combined.

APPENDIX 2. Duncan's multiple range analysis for 10 treatments from trials 1 and 2

Mean percentage of styroblock pieces supporting growth of all fungal and algal species combined and all fungal species combined from trials 1 and 2.

Trial 1

Treatment	Compound or sanitizing method	Mean percentage of styroblock pieces supporting: Fungi and algae[1]		Fungi only[1]	
1.2a	Control	34.5	a	26.5	ab
1.1a	Control	28.6	a	20.0	a
11.1a	*De-Moss*	19.1	ab	4.5	cd
12.2a	*Ivory* soap	19.0	ab	7.5	bcd
10.1a	Sodium metabisulphite	18.3	ab	2.5	d
12.3a	*Ivory* soap	15.6	ab	0.5	bcd
9.2a	Bleach	15.0	ab	10.0	abc
10.2a	Sodium metabisulphite	13.4	ab	6.0	d
11.2a	*De-Moss*	12.9	ab	3.5	d
13.1a	Hydrogen peroxide	0.1	b	0.0	d

Trial 2

Treatment	Compound or sanitizing method	Mean percentage of styroblock pieces supporting: Fungi and algae[1]		Fungi only[1]	
2.1b	Control	45.5	a	33.5	a
1.1b	Control	29.5	b	15.7	b
8.2b	*De-Moss*	14.0	c	5.0	c
8.3b	*De-Moss*	13.5	c	5.5	c
9.2b	Hydrogen peroxide	12.5	c	5.3	c
9.3b	Hydrogen peroxide	12.5	c	5.7	c
8.4b	*De-Moss*	10.5	c	2.2	c
11.1b	Steam	6.0	c	3.0	c
11.2b	Steam	3.5	c	3.8	c
11.3b	Steam	0.5	c	0.3	c

[1] Treatments with the same letter (a,b,c,d) have means that are not significantly different at the 1% level of significance.

Mean percentage of styroblock pieces supporting growth of individual fungal species and all algae from trials 1 and 2.

Trial 1

Treatment	Compound or sanitizing method	Mean percentage of styroblock pieces supporting							
		Fusarium[1]		*Phoma*[1]		*Cylindrocarpon*[1]		Algae[1]	
1.2a	Control	38.7	a	26.9	ab	5.3	a	69.2	a
1.1a	Control	25.7	ab	28.8	a	2.5	a	59.0	ab
11.1a	*De-Moss*	0.0	c	4.0	cd	6.5	a	66.0	ab
12.2a	*Ivory* soap	5.0	bc	17.5	bc	0.0	a	53.5	ab
10.1a	Sodium metabisulphite	0.0	c	1.5	d	0.0	a	71.5	ab
12.3a	*Ivory* soap	2.5	bc	19.0	b	0.0	a	41.0	abc
9.2a	Bleach	6.0	bc	27.5	ab	1.0	a	34.0	bc
10.2a	Sodium metabisulphite	0.0	c	0.0	d	0.0	a	53.5	ab
11.2a	*De-Moss*	0.5	bc	4.0	cd	0.0	a	44.0	ab
13.1a	Hydrogen peroxide	0.0	c	0.5	d	0.0	a	0.0	c

Trial 2

Treatment	Compound or sanitizing method	Mean percentage of styroblock pieces supporting							
		Fusarium[1]		*Phoma*[1]		*Cylindrocarpon*[1]		Algae[1]	
1.2b	Control	41.5	a	59.0	a	0.0	b	82.0	a
1.1b	Control	7.5	b	33.0	b	6.5	a	70.5	a
8.2b	*De-Moss*	4.0	b	9.0	c	1.0	b	40.5	b
8.3b	*De-Moss*	1.0	b	13.5	c	2.5	b	38.5	b
9.2b	Hydrogen peroxide	1.5	b	3.0	c	11.5	a	34.0	bc
9.3b	Hydrogen peroxide	0.0	b	5.0	c	12.0	a	33.0	bc
8.4b	*De-Moss*	1.0	b	4.0	c	1.5	b	35.0	bc
11.1b	Steam	6.5	b	2.5	c	0.0	b	18.5	bc
11.2b	Steam	3.0	b	8.0	c	0.0	b	3.0	c
11.3b	Steam	0.0	b	1.0	c	0.0	b	1.5	c

[1] Treatments with the same letter (a,b,c,d) have means that are not significantly different at the 1% level of significance.

APPENDIX 3. Duncan's multiple range analysis for all treatments from trial 3

Mean percentage of styroblock pieces supporting growth of all fungal and algal species combined and all fungal species combined (A) and individual fungal species and all algae (B) from trial 3

A

Treatment	Compound or sanitizing method	Mean percentage of styroblock pieces supporting:	
		Fungi and algae[1]	Fungi only[1]
8.1c	Iodine	23.6 a	19.8 ab
1.1c	Control	23.3 a	11.7 abc
2.1c	*De-Moss*	21.6 a	21.5 a
3.1c	*Ivory* soap	19.4 a	18.3 ab
1.2c	Control	18.5 a	24.7 a
4.1c	Bleach	17.4 a	14.2 abc
6.1c	Hydrogen peroxide	8.5 ab	3.8 bc
7.1c	Steam	2.6 bc	3.5 bc
5.1c	Sodium metabisulphite	0.0 bc	0.0 c
9.1c	Sulphur dioxide	0.0 c	0.0 c

B

Treatment	Compound or sanitizing method	Mean percentage of styroblock pieces supporting			
		Fusarium[1]	*Phoma*[1]	*Cylindrocarpon*[1]	Algae[1]
1.2c	Control	8.5 a	0.0 a	65.5 a	0.0 c
1.1c	Control	6.5 ab	0.0 a	28.5 c	58.0 a
8.1c	Iodine	6.5 ab	0.5 a	52.5 ab	35.0 b
2.1c	*De-Moss*	4.5 ab	0.0 a	60.0 a	22.0 bc
7.1c	Steam	4.0 ab	3.5 a	3.0 d	0.0 c
3.1c	*Ivory* soap	3.0 ab	0.0 a	52.0 ab	22.5 bc
4.1c	Bleach	2.0 ab	2.5 a	38.0 bc	27.0 bc
6.1c	Hydrogen peroxide	0.5 b	1.0 a	10.0 d	22.5 c
5.1c	Sodium metabisulphite	0.0 b	0.0 a	0.0 d	0.0 c
9.1c	Sulphur dioxide	0.0 b	0.0 a	0.0 d	0.0 c

[1] Treatments with the same letter (a,b,c,d) have means that are not significantly different at the 1% level of significance.

APPENDIX 4. Duncan's multiple range analysis for trial 3 phytotoxicity tests

Mean number of surviving lettuce plants per treatment at the end of 5 weeks. Treatments are listed in order of decreasing plant viability.

Treatment	Compound or sanitizing methods	Mean number of surviving plants[1]	
1.2c	Control	8.7	a
2.1c	*De-Moss*	8.6	a
9.1c	Sulphur dioxide	8.5	a
3.1c	*Ivory* soap	8.1	ab
5.1c	Sodium metabisulphite	6.9	bc
7.1c	Steam	5.8	cd
4.1c	Bleach	5.4	cd
8.1c	Iodine	5.1	d
6.1c	Hydrogen peroxide	5.0	d
1.1c	Control	4.9	d

Mean fresh weights and oven-dried weights of lettuce plants at the end of 5 weeks.

Treatment	Compound or sanitizing method	Fresh weight (g)[1]		Dried weight (g)[1]	
4c	*Ivory* soap	0.3027	a	0.0234	a
8c	Steam	0.2936	a	0.0197	a
10c	Sulphur dioxide	0.1208	b	0.0096	b
3c	*De-Moss*	0.0873	bc	0.0089	bc
2c	Control	0.0811	bc	0.0048	bcd
5c	Bleach	0.0715	bc	0.0030	cd
1c	Control	0.0492	bc	0.0030	cd
6c	Sodium metabisulphite	0.0431	bc	0.0029	cd
7c	Hydrogen peroxide	0.0400	bc	0.0033	cd
9c	Iodine	0.0046	c	0.0008	d

[1] Treatments with the same letter (a,b,c,d) have means that are not significantly different at the 1% level of significance.